

Assessment of a selective medium for the isolation of *Neisseria gonorrhoeae*

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SUMMARY Specimens of urethral pus from 312 men with gonorrhoea were diluted and inoculated on to non-selective and selective media, the latter containing vancomycin, colistin, nystatin, and trimethoprim. Although three (1%) isolates of *Neisseria gonorrhoeae* were inhibited by trimethoprim 4 mg/l and 10 by vancomycin 2 mg/l, only two strains failed to grow on a selective medium from large inocula but eight strains sensitive to vancomycin failed to grow on a selective medium from a light inoculum. These few failures do not appear to negate the value of the selective medium.

Introduction

Various antibiotics have been used in selective media for the isolation of the gonococcus from sites with a heavy, normal, or contaminating flora. We have used a medium containing vancomycin, colistin, and trimethoprim with success for a number of years (Phillips *et al.*, 1972). It has been suggested, however, that both vancomycin and trimethoprim inhibit a considerable number of strains of *N. gonorrhoeae* and are therefore unacceptable (Cross *et al.*, 1971; Brorson *et al.*, 1973). We, therefore, decided to investigate the *in-vitro* activity of trimethoprim and vancomycin on the gonococcus and to assess results in the light of the success or failure of primary isolation of the same strains on non-selective and selective media.

Materials and methods

ISOLATES OF *NEISSERIA GONORRHOEAE*

Samples of pus from male patients with gonorrhoea attending the Department of Genitourinary Medicine at St Thomas's Hospital were collected on a plain cottonwool swab, which was immediately placed in 1 ml of nutrient broth (Southern Group Laboratories) containing 10% saponin-lysed horse blood. Standard loopfuls (0.007 ml) of the neat broth (large inoculum) and a dilution of 1/1000 (small inoculum) were used to inoculate in parallel both a non-selective

and selective medium. The non-selective medium was lysed-blood agar (Oxoid DST agar CM 261 plus 5% saponin-lysed horse blood), and the selective medium was 10% lysed-blood agar (Difco heart infusion agar 0044-01) containing vancomycin 3 mg/l, colistin sulphomethate 100 units/ml, nystatin 12.5 units/ml, and trimethoprim 5 mg/l, similar (except in the agar base) to VCNT medium, which we have used for many years for the routine isolation of gonococci (Phillips *et al.*, 1972). We had previously shown that the two different media performed similarly when antibiotics were not incorporated, and we, thus, used different agar as described for purely operational reasons.

MINIMUM INHIBITORY CONCENTRATIONS

Minimum inhibitory concentrations (MICs) were determined for vancomycin and trimethoprim by a lysed-blood agar (Oxoid DST) dilution method described previously (Phillips *et al.*, 1970; Lawrence *et al.*, 1973).

The inoculum was prepared from isolates cultured on a non-selective medium and consisted of 10^4-5 colony-forming units.

Results

A total of 312 isolates was studied, comprising all those that grew on primary isolation on the non-selective medium during the period of study.

Table 1 shows the distribution of sensitivity of isolates to vancomycin and trimethoprim. Ten (3%) isolates were inhibited by vancomycin 2 mg/l or less and three (1%) by trimethoprim 4 mg/l. No isolate was highly sensitive to both agents.

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Table 1 Susceptibility of 312 isolates of *Neisseria gonorrhoeae* to vancomycin and trimethoprim

Antibiotics	% of isolates with MICs (mg/l) of								
	1	2	4	8	16	32	64	128	>128
Vancomycin	0.5	2.5	10	32	36	16	3		
Trimethoprim			1	2	19	43	22	8	5

Table 2 shows the results of primary cultures of large and small inocula (the latter varying between about 10 and 100 colony-forming units) on non-selective and selective media. All three organisms with a MIC of 4 mg/l for trimethoprim grew from both large and small inocula on the selective medium. Among the remaining isolates two failed to grow even from large inocula while eight failed to grow and two were considerably inhibited from small inocula on the selective medium. Examination of the results for vancomycin shows that these failures were associated with vancomycin sensitivity. Two isolates with MICs of 1 mg/l for vancomycin completely failed to grow on the selective medium. A further eight isolates, with MICs of 2 mg/l, grew well from large inocula but either failed to grow completely (six isolates) or were very considerably inhibited (two isolates) from small inocula. All isolates with MICs of 4 mg/l or more for vancomycin grew well from large and small inocula, regardless of the MICs for trimethoprim.

Discussion

Selective media are vital for the microbiological diagnosis of gonorrhoea in women, but the assessment of the performance of these media is difficult because of the unreliability of direct Gram stains of material from the female genital tract and the lack, therefore, of a basis for comparison. Gram

stains of specimens of urethral pus from men are, in contrast, a very good means of diagnosing gonorrhoea, and samples from men, therefore, offer a better method of assessing the performance of selective media. The number of gonococci in specimens of urethral pus from men, however, is usually very large, and we, therefore, decided that a more stringent test of the inhibitory activity of a selective medium might be provided by the use of inocula prepared from diluted specimens of pus.

The other means of examining the probable effects of antibiotics in selective media is to determine the MICs of the antibiotics. Because the conditions suitable for sensitivity testing differ from those for isolation, discrepancies might be expected.

Our results show that gonococci that are sensitive to trimethoprim and vancomycin are uncommon in patients attending the Department of Genitourinary Medicine at St Thomas's Hospital. Only 3% of strains were sensitive to vancomycin 2 mg/l, a similar result to that of 9% of strains sensitive to vancomycin 3 mg/l in our last study (Phillips *et al.*, 1972), and only 1% were sensitive to trimethoprim 4 mg/l compared with 12% to trimethoprim 3 mg/l in our last study which shows a possible increase in resistance. Results of parallel primary isolation on non-selective and selective media, however, show that this degree of trimethoprim sensitivity did not prevent the isolation of *N. gonorrhoeae* from either large or small inocula of the few strains in this group. Vancomycin sensitivity was important, however, in that both of the isolates that were highly sensitive (MIC=1 mg/l) failed to grow on the selective medium, while eight slightly less sensitive isolates (MIC=2 mg/l) either failed to grow or were considerably inhibited in small inocula, although they grew perfectly well from larger inocula. It is of interest that only one of the isolates out of the 312

Table 2 Results of parallel cultures of 312 isolates of *N. gonorrhoeae* on non-selective and selective media containing vancomycin 3 mg/l and trimethoprim 5 mg/l

Antibiotic	MIC (mg/l)	Inoculum	No. of isolates giving growth on media			
			Non-selective	Selective		
				++	+	-
Trimethoprim	4	large and small	3	3	0	0
	≥8	large	309	307	0	2
		small	309	299	2	8
Vancomycin	1	large and small	2	0	0	2
	2	large	8	8	0	0
		small	8	0	2	6
	≥4	large and small	302	302	0	0

+ + Same amount of growth as on non-selective medium

+ Less growth than on non-selective medium

- No growth

was not detected in the routine diagnostic section of our laboratory, which uses selective plates inoculated in the clinic with undiluted specimens of urethral pus. These results contrast with those of Cross *et al.* (1971), who found that 8% of primary isolations failed, and of Brorson *et al.* (1973), who lost 10% of primary isolations because of the high level of sensitivity to vancomycin among gonococci.

Thus, the use of our selective medium, consisting of heart-infusion agar containing 10% lysed horse blood, vancomycin 3 mg/l, and trimethoprim 5 mg/l in addition to colistin and nystatin (VCNT), seems to be fully justified in our clinic for the isolation of gonococci from men and, by inference, from women.

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